



#17

PATENT**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application Of:)	Group Art Unit: 1642
)	
NICOLAIDES et al.)	BOX: MISSING PARTS
)	
Serial No.: 09/853,646)	
)	Docket No. 001107.00138
Filed: May 14, 2001)	
)	
For: A METHOD FOR GENERATING)	
HYPERMUTABLE ORGANISMS)	

AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231
Attn: Box Missing Parts

Dear Sir:

In response to the Notice to File Missing Parts Of Non-provisional Application, dated August 9, 2001, the Applicants are hereby submitting the following amendments for entry into record. No fees are believed to be due. However, if such a fee is found to be necessary, please charge to Deposit Account No. 19-0733.

IN THE SPECIFICATION:

On pages 34-35, first full paragraph, please substitute the following:

Plasmids.

The full-length wildtype *hMLH1* cDNA was obtained from a human Hela cDNA library as described (18). A MLH1 cDNA containing a termination condon was obtained via RTPCR from the patient in which the mutation was discovered (24). The cDNA fragments were cloned into the XhoI site of the pCEP9 vector (Invitrogen), which contains CMV promoter followed by an SV40 polyadenylation signal (8) and a gene, which encodes for neomycin resistance. The pC9MLH1 vector produces the full-length

function MLH1 protein, while the pC9MLH1 stop produces the non-functional truncated MLH1 polypeptide. The polyPNP and PNP vectors are described in Fig. 4. The polyPNP contains a 21 base out-of-frame polyA tract inserted after codon 2 of the bacterial PNP gene which results in a truncated polypeptide (Sorscher, EJ, et.al. Gene Therapy 1:233-238, 1994). The polyPNP contains a 20base in-frame polyA tract inserted after condon 2 of the bacterial PNP gene which results in a full-length functionally active PNP protein. Both the polyPNP and PNP gene have a hemagglutinin (HA) epitope fused in-frame at the C-terminus followed by a termination codon. The polyPNP and the PNP gene was constructed by polymerase cahin reaction using a sense primer : 5'-
ccaagcttagaccaccatggcaaaaaaaaaaaaaaaaaaaatcgctacccacacattaatgc-3' (SEQ ID NO: 1), where the polyA tract is underlined while the primer for PNP contains 1 less A in the polyA tract. The antisense primer for both constructs is
5'ataagaatcgggccgctatccttagctagcgtaatctggaacatcgtaagcgtaatctggaacatcgctactctttatcgcccagcag-3' (SEQ ID NO: 2). DH5 α bacterial DNA was used a template for amplification. The modified PNP gene was produced by amplification USING 95 $^{\circ}$ c FOR 30 SEC, 54 $^{\circ}$ c FOR 1 MINUTE, 72 $^{\circ}$ c for 1 min for 25 cycles in buffers as previously described (19). Amplified genomic inserts were cloned into T-tailed vectors (TA cloning, Invitrogen) and recombinant clones were sequenced to identify vectors with correct nucleotide sequences. PNP fragments were then subcloned into the KpnI-XhoI sites of the pCeP4 vector (Invitrogen) using sites from the TA cloning vector polylinker. Recombinant PNP expression vectors were sequenced to ensure sequence authenticity using internal primer sequences.

On page 9, the second full paragraph please substitute the following paragraph:

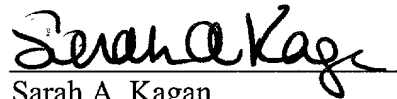
Figure 7. Diagram of the genetically altered purine phosphorlyase (PNP) gene with an out-of-frame poly A tract inserted in the N-terminus (referred to as polyPNP) (SEQ ID NO: 3). This gene

encodes for a non-functional PNP gene. When the polyA tract is randomly altered by a defective MMR, the tract is shifted and allows for the production of a functional PNP gene. PNP can convert the non-toxic prodrug 9-(β -D-2-deoxyerythropentofuranosyl)-6-methyl- purine (referred to as MPD) to the toxic 6-methyl purine analog (referred to as (MP)). The construct has a hemagglutinin (HA) tag at the C-terminus for western blot analysis. A control construct with an in-frame polyA tract encoding a full-length polypeptide (SEQ ID NO: 4) is shown on the bottom.

Respectfully submitted,

October 26, 2001

By:



Sarah A. Kagan

Registration No. 32,141

Banner & Witcoff, LTD.
Eleventh Floor
1001 G Street, N.W.
Washington, D.C. 2001
(202)508-9100

09/16/2004

MARKED-UP COPY OF AMENDMENT

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